AD-A187 388 EFFECT OF ELECTRIC FIELDS ON MEMBRANE BOUND (NAK)-ATPASE(U) JOHNS HOPKINS UNIV BALTIMORE MD DEPT OF BIOLOGICAL CHEMISTRY T Y TSONG 06 NOV 87 1/1

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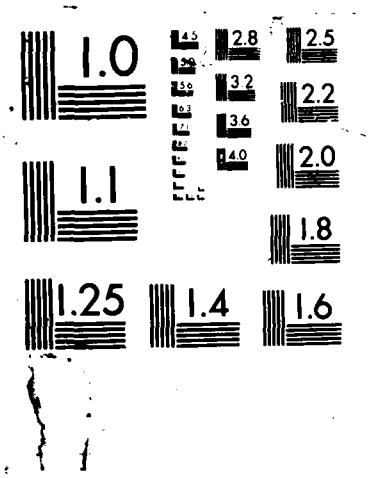
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A protein of a cell membrane is structurally sensitive to an electric field because of its charges, helix dipoles and polarizability. When such a protein is exposed to an applied oscillating electric field, the conformational oscillation of the protein can be coupled to driving an endergonic reaction. We have investigated how such an interaction between the (Na,K)-ATPase of human erythrocytes and an a.c. field leads to active pumping of K^+ and Na^+ against their respective concentration gradient. Experiments done in this past year establish that the optimum frequency of Rb^+ (an equivalent of K^+) occurs at 1.0 kHz and that for Na^+ efflux at 1 MHz or higher. The optimum voltage for both pumps is 20 V/cm. An electroconformational coupling model is proposed and the above data analyzed according to the model. In the continuation years we will study the relationship between stimulating voltage and optimum frequency, and vise versa. Its molecular mechanisms will be investigated and more quantitative analysis performed. biochemistry, biophysics

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Tsong, Tian Y.

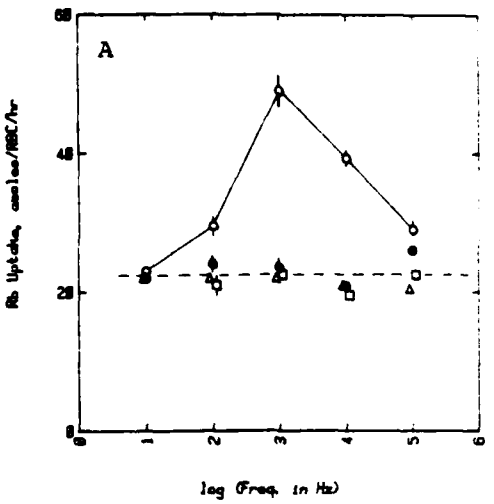
INTRODUCTION

The main objective of this project is to investigate how an electric field interacts with living cells or tissues. Specifically, we have been studying effect of electric fields on ion pumping activity of [Na,K]-ATPase. Our previous result shows that this enzyme can be activated to pump ions against their respective concentration gradient. However, data appropriate for interpretation have been limited to a.c. induced Rb^+ uptake. It was shown that, at 3°C, with a 20 V/cm a.c. field the stimulated Rb^+ uptake by human erythrocytes reached a value of 22 ion per sec per enzyme when the frequency of the a.c. field was 1 kHz. It was also shown that this activity was completely inhibited by ouabain, a potent inhibitor of [Na,K]-ATPase. However, data were not complete and we were not certain whether 1 kHz was the optimum frequency for the activation of [Na,K]-ATPase. Another uncertainty was that no Na^+ pumping activity was stimulated by the a.c. field. This inability to stimulate the Na^+ pump made our result suspect of experimental artifacts. Most reviewers agree that if we can demonstrate unequivocally the activation of the Na^+ and the K^+ pumps by an a.c. field it would be a significant finding for understanding mechanisms of energy and signal transductions in cells. The most pressing goal for us last year was to refine our experiment to answer these questions.

PROGRESS REPORT

1. Maximum Rb^+ uptake at 1.0 kHz: Our new experiments on the a.c. stimulated Rb^+ uptake by human erythrocytes confirms the result of Serpersu & Tsong (J. Membrane Biol. 74, 191-201, 1983; J. Biol. Chem. 259, 7155-7162, 1984). In addition, experiments have been extended to include 200 Hz and 7000 Hz. In repeated measurements, the uptakes at 200 Hz and 7000 Hz were always less than that measured at 1.0 kHz. Figs. IA and IB compare the result of Serpersu & Tsong and that obtained recently by Liu & Tsong (1987, to be published). Data in Fig. IB greatly strengthen our confidence that the observed voltage stimulated Rb^+ uptake is a real phenomenon and is not due to unsolicited experimental artifacts.

2. Activation of Na^+ Pump: But the most crucial result we have obtained in this period is the activation of the Na^+ pump which, again, was inhibited by ouabain. As we suspected, Na^+ pump was activated at another frequency, 1 MHz. Whether 1 MHz is the optimum frequency for activating this pump remains unknown. Our present instrumentation can not reach frequency above 1 MHz. In the next year's budget, funds for purchasing a high frequency voltage generator (which can reach 20 MHz) are included, and we will be able to answer this question quickly. What is interesting is that the maximum stimulated Na^+ efflux at 1 MHz using 20 V/cm field was about 33 ion per sec per enzyme, 1.5 times greater than that of the maximum Rb^+ uptake at identical field strength, but at 1.0 kHz. This observation suggests that 1 MHz is close to, if not, the frequency optimum of the Na^+ pump activation. Now that both Na^+ and K^+ pumps



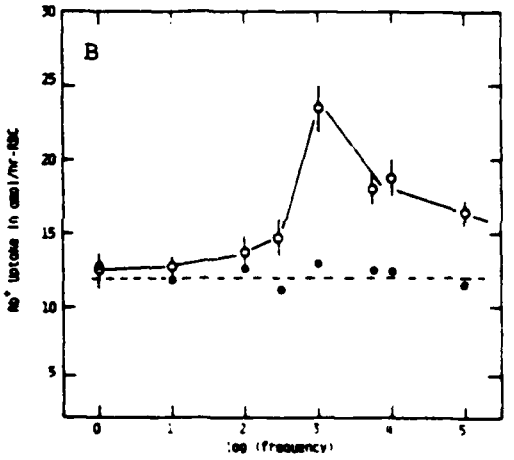
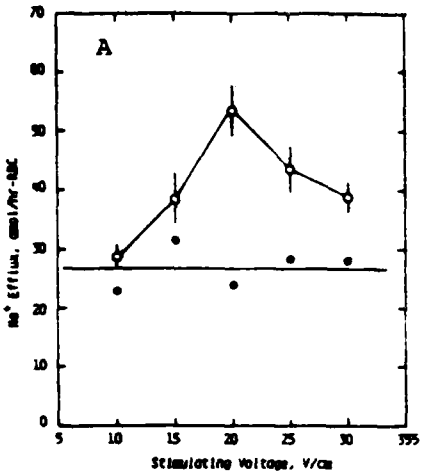


Fig. I Voltage induced Rb^+ uptake by human erythrocytes via [Na,K]-ATPase: Frequency dependence.

A. Data of Serpersu & Tsong [J. Membrane Biol. 74, 191-201 (1983)]. For the experimental conditions please see the original paper. Open circles: data from samples stimulated with 20 V/cm a.c. field at different frequencies. Other symbols are data from controls.

B. Data by Liu & Tsong [This work, to be published]. Measurements were extended to include 200 Hz and 7000 Hz. The result is similar to that shown in A, except that the general level of stimulated activity is lower. This is due to the use of red cells from different individual in this experiment. Other experimental conditions are identical to that used in A. The optimum frequency remains at 1 kHz.



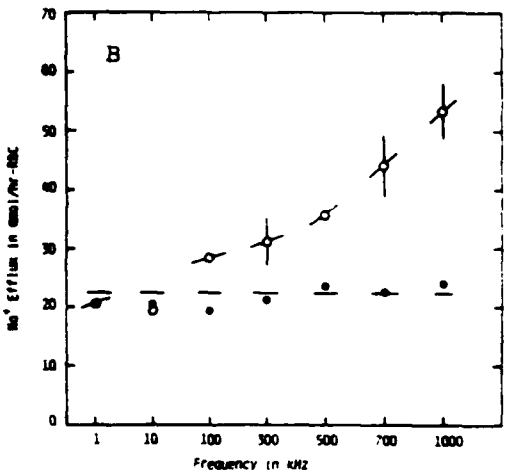


Fig. II Voltage induced Na^+ efflux in human erythrocytes via [Na,K]-ATPase.

A. Dependence on field strength when an a.c. frequency of 1 MHz was used. The maximum occurs at 20 V/cm, identical to that obtained for Rb^+ uptake using 1 kHz a.c. field.

B. Frequency dependence using an a.c. field of 20 V/cm. Note that the X-axis is neither in linear nor in log scale. It is chosen only for presenting result conveniently. When log-scale is used, as in Fig. IB, the experimental line will rise sharply. Experimental conditions are identical to that given in Serpersu & Tsong's paper.

have been activated by the a.c. field, any reservation about these results can be dismissed. Figs. IIA and IIB give the result of a typical experiment with the Na^+ pump.

3. Model Analysis: In the June of 1985, I proposed, at the meeting of the Bioelectrochemical Society in Bologna Italy, a model based on the idea that an applied electric field can induce protein conformational transitions which when coupled to ligand binding and dissociation steps can drive an endergonic reaction. Last year, in collaboration with Drs. Astumian, Westerhoff and Chen of NIH, we have done more analysis. The results of model simulations indicate that the concept of the electroconformational coupling is a viable mechanism for the transduction of energy and signal from an oscillating electric field. Our current interest is to investigate whether a stationary transmembrane potential can be modulated to become locally oscillatory by an internal mechanism of an enzyme for energy and signal transductions. We are investigating the possibility that F_o subunit of mitochondrial ATPase is a potential modulating subunit of the enzyme.

PUBLICATIONS

1. Tsong, T.Y. & Astumian, R.D. (1986). Absorption and conversion of electric field energy by membrane bound ATPases. Bioelectrochem. Bioenerg. 15, 457-476.

2. Westerhoff, H., Tsong, T.Y., Chock, P.B., Chen, Y. & Astumian, R.D. (1986). How enzyme can capture and transmit free energy from an oscillating electric field. Proc. Natl. Acad. Sci. USA 83, 4734-4738.

3. Astumian, R.D., Chock, P.B., Tsong, T.Y., Chen, Y.D. & Westerhoff, H. (1987). Can free energy be transduced from electric noise? Proc. Natl. Acad. Sci. USA 84, 434-438.

4. Tsong, T.Y. & Astumian, R.D. (1987). Electroconformational coupling and membrane protein function. Prog. Biophys. Mole. Biol. In press.

5. Tsong, T.Y. & Astumian, R.D. (1988). Electroconformational coupling: An efficient mechanism for energy transduction by membrane-bound ATPases. Ann. Rev. Physiol. 50. In press.

6. Tsong, T.Y., Chauvin, F. & Astumian, R.D. (1987). Interaction of membrane proteins with static and dynamic electric fields via electroconformational coupling. In "Mechanistic Approaches to Interactions of Electromagnetic Fields with Living Systems", M. Blank & E. Findl, eds., Plenum Publ. Corp. In press.

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